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Concerning the Occurrence of Free Pentoses in Plants

CONCERNING THE OCCURRENCE OF FREE PENTOSES IN PLANTS

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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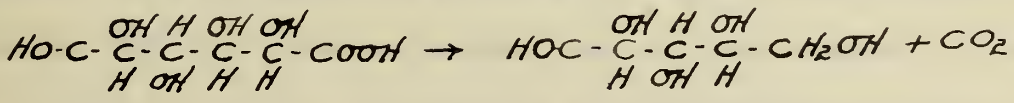
I

Introduction and History.

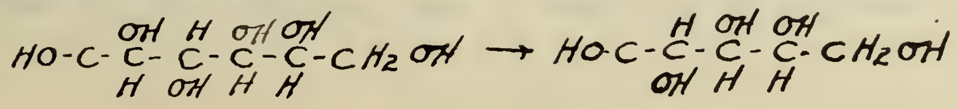
That pentoses occur in plants in the combined state has been a recognized fact since the first study of the existence of sugars in plants. According to Armstrong (1), pentoses are never found as simple sugars but as polysaccharides of high molecular weight and as complex glucosides which are present essentially as skeletal and not as food products; and are, therefore, outside the range of the ordinary plant enzymes. He also points out that the simple sugars more than then complex carbohydrates exert a preponderating influence on pentose formation, the amount of pentoses increasing during the day and decreasing with increase of moisture.

Spoehr (2) brings out the theory of the formation of pentoses from such substances as glucuronic acid. He states that in the disaccharides and polysaccharides, the CH_2OH group is the first to be affected by chemical change with the result that glucuronic acid is formed. Since a general property of these acids is the splitting off of CO_2 from the carboxyl group, glucuronic acid would form l-xylose:

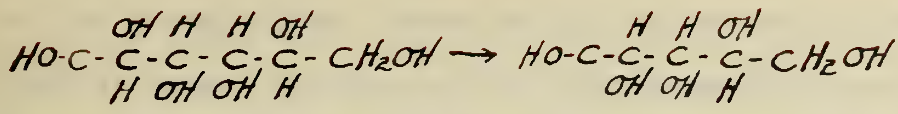


$$HO\text{-}C\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{H}{\underset{OH}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}COOH \rightarrow HOC\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{H}{\underset{OH}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}CH_2OH + CO_2$$

Furthermore, if pentoses are derived from a direct oxidation of hexoses, d-glucose would yield d-arabinose and d-galactose give d-lyxose:



$$HO\text{-}C\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{H}{\underset{OH}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}CH_2OH \rightarrow HO\text{-}C\text{-}\overset{H}{\underset{OH}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}\overset{OH}{\underset{H}{C}}\text{-}CH_2OH$$



$$HO\text{-}C\text{-}\underset{H}{\overset{OH}{C}}\text{-}\underset{OH}{\overset{H}{C}}\text{-}\underset{OH}{\overset{H}{C}}\text{-}\underset{H}{\overset{OH}{C}}\text{-}CH_2OH \rightarrow HO\text{-}C\text{-}\underset{OH}{\overset{H}{C}}\text{-}\underset{OH}{\overset{H}{C}}\text{-}\underset{H}{\overset{OH}{C}}\text{-}CH_2OH$$

It is a fact, however, that in nature d-glucose is almost always found with l-xylose, and that d-galactose is associated with l-arabinose and that the pentose sugars thus found are of the l- and not of the d- series.

It was not until 1914, however, that Davis and Sawyer (3) first brought out the theory that pentoses existed in the free state. They based their conclusions on the fact that a non-fermentable reducing sugar residue was obtained after fermentation with yeast and also that by running the regular Kröber and Tollens determination of pentoses by distillation with hydrochloric acid, a greater precipitate was obtained with phloroglucin than could be accounted for as due to hexoses. They also show that the furfural-like compounds produced from other sugars is of a very small significance in the majority of cases.

However, Davis used a 1% solution of ammonium hydroxide, Sp. Gr..88, for neutralizing the organic acids in the refluxing process of extracting the sugars from the plant material. This seems to leave his work open to criticism on account of the well known isomerizing effect of weak alkalies. This was pointed out as early as 1895 by Lobry de Bruyn (4). Also, the extensive researches of Nef (5) have indicated that a great number of products may be formed by action of alkalies on the monosaccharides in the presence of oxidizing agents and that the nature of the products depends largely on the concentration of those substances. Working with ammonia, he took 24.5 gm. of dextrose in a closed vessel with

200 cc. of 1 equivalent ammonium hydroxide and allowed the solution to stand 74 days. At the end of that time the solution was colored yellow and showed C 4.9 and 85.2% sugar. Also, 10 cc. of the solution needed 28.3 cc. of N/10 HCl for neutralization instead of the 27.4 cc. calculated quantity. The 8% loss of ammonia is probably due to the saturation of the C_6 acids which were formed. Accordingly, ammonium hydroxide must act like traces of caustic alkali upon sugar solutions.

Ruff (6) showed that by using ferric salts with hydrogen peroxide, some oxidation or degradation takes place with the formation of d-arabinose, the ferric salts acting as a catalytic agent.

Therefore, due to Davis' method of treatment with ammonium hydroxide and the use of iron vessels, perhaps enough isomerization of levulose to glutose or some other non-fermentable sugar could have taken place to give the reducing residue. Furthermore, it is probable that some intramolecular change in the sugar or some degradation could have given rise to a product which leads to the production of more furfural than the original hexoses.

II

Theoretical and Experimental.

The experiment, therefore, was essentially a duplication of Davis' work, using approximately 10 gm. of a mixture of sucrose and invert sugar; endeavoring to ascertain whether any errors could be introduced due to the various influences as mentioned in the introduction to this paper.

Preparation of Solutions.- Twenty grams of pure sucrose was dissolved in 220 cc. of water. 100 cc. of this solution was inverted and made up to 220 cc. (solution A) and 100 cc. was simply made up to 220 cc. (solution B). In this manner, by using 100 cc. of both solutions A and B, approximately 10 gm. of the sugar would be used in a determination.

Refluxing with Ammonium Hydroxide.- The next step consisted in refluxing for eighteen hours, 100 cc. of each solution A and B in 1500 cc. of boiling alcohol to which 5 cc. of concentrated ammonium hydroxide had been added. The resulting solution was then concentrated to 150 cc., made up to 500 cc,, cleared with basic lead, and washed almost to 2000 cc. The excess lead was removed with sodium carbonate and the solution made up to 2 liters.

Determination of Pentoses by Distillation - Fifty cc. portions of the above solution was then distilled with HCl according to the regular Krober and Tollen's (7) method of analysis and the precipitate resulting from the addition of phloroglucinol was weighed. The result of this first run showed a phloroglucid precipitate of .0191 gm. and .0188 gm.

In carrying out the last distillation, the rate was kept as high as possible (30 cc. every 10 min.) since, according to Van Haarst and Olivier (8), except in the case of l-arabinose, the most complete distillation of the furfural is obtained when the time is short, continued boiling with HCl tending to decompose the furfural.

After the addition of the phloroglucinol, the solution was allowed to stand over night in order to insure complete precipitation. If allowed to stand for a longer time a heavier precipitate is formed which, according to the work of Cunningham and Dorie (9), is w-hydroxymethylfurfuraldehyde. They state that this compound is formed by the action of HCl acid on hexoses, starch, and celluloses, but that owing to its slow formation it does not interfere with the accuracy of the pentose determination as long as aniline acetate is used as an indicator of complete distillation. This compound also differs from the phloroglucid in that the latter is black while the former is brownish-red.

Removal of Hexoses and Determination of Pentoses by Reduction Method.- After the above determination was finished, 1000 cc portions of the 2 liter solution was concentrated in a vacuum to 250 cc. and 50 cc. portions fermented for three to five weeks with Sach Ceviseae, cleared with alumina cream, and washed to 100 cc. with boiling water. A regular Browne-Morris copper oxide reduction was run on 50 cc. portions of the solutions.

Results:

cc. $Na_2S_2O_3$	CuO	Arabinose mg.
3.4	.0255	9.2
4.32	.0324	12.0

The next step was a blank Krober-Tollen's distillation on 50cc. portions of a 2 liter solution made by simply diluting 100 cc of each of the two solutions A and B. The results obtained gave a precipitate of .0078 gm. and .0085 gm.

A reduction determination was also made on 50 cc. portions of the above solution, after three to five weeks fermentation, but the results showed no reduction of the copper oxide.

Effect of Concentration.- In the vacuum concentration as carried out above, a slow stream of air was allowed to bubble thru the solution in order to prevent bumping. As this passage of air might result in a slight oxidation to pentose sugars, a distillation was carried out on solutions which had been concentrated in a vacuum without the passage of the air. The results obtained in this way showed a precipitation of .0165 gm. and .0162 gm.

In order to ascertain whether the ammonium hydroxide had an effect on the production of pentoses if allowed to remain in contact with sugar for a shorter time, another determination was made on solutions in which there was no refluxing with the ammonium hydroxide. Here a precipitate was obtained which amounted to .0097 gm. and .0091 gm. Furthermore, the w-hydroxymethylfurfuraldehyde precipitate was very small as compared with the other determinations.

III

Recovery of Iodide Salts.

In the copper oxide reduction method as carried out above and especially in using the Peter's modification method, large quantities of potassium iodide was used. Because of the shortage and cost of this product an attempt was made to recover it from the titration residues.

Stephenson (10) showed that the free iodine could be obtained by treating the solution with nitric acid and hydrochloric acid. Trossian (11) also showed that the iodine could be obtained by treating the residues with hydrogen peroxide. Nothing could be found, however, in regard to the recovery of the iodine in the form of the iodide.

The first step in the recovery consisted in the partial neutralization of the solution containing the sulphates and tartrates of sodium and potassium, the iodide of copper, the sodium thiosulphate, and starch, with sodium hydroxide, leaving the solution only slightly acid in order to prevent fhe formation of iodates. By passing H_2S into this solution, the copper sulphide is precipitated out, carrying the starch down with it. The solution was filtered, leaving a practically clear solution of the sulphate and iodide salts of sodium and potassium. By evaporating this filtrate these salts crystallize out, giving white crystals of sodium and potassium iodides and sulphates. Since the iodides are soluble in alcohol while the sulphates are not, the former were dissolved in alcohol, best by using a Soxhlet extractor for several hours. The sodium and potassium iodides were then crystallized from the alcoholic

solution.

In order to insure that no free iodine is present, the filtrate, after the CuS has been precipitated out, should be washed with carbon bisulphide in a separatory funnel, the carbon bisulphide dissolving any free iodine.

The above method of recovery gives a sufficiently pure product of sodium and potassium iodides to enable one to use these salts over again in the thiosulphate titrations.

IV

Summary.

1. In a recent paper, pentoses have been reported as occuring in the free state. The evidence for their presence is based on the facts (1) that after fermentation of the hexoses with brewer's yeast a non-fermentable residue remains and (2) more furfural is produced by distillation of a portion of the original solution with dilute hydrochloric acid than could be accounted for from the hexose alone. The extraction of the sugars involved refluxing with ammoniacal alcohol and extensive concentrations.

2. It is shown in the present paper that a reproduction of this process of extraction and concentration with pure sucrose and invert sugar gives results comparable to those reported. Hence, the apparent free pentoses are probably derived from the hexoses.

3. In the absence of ammonia some furfural is obtained, but no residue, non-fermentable with brewer's yeast.

4. The formation of the apparent pentoses is due primarily to the action of air in contact with the ammoniacal solution of the sugars.

V
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